by the direct method and refined by block-diagonal least-squares technique on FACOM M-340R computer to R and R_w = 0.045 and 0.066, respectively, for 2472 observed reflections with $|F_o| > 3\sigma(F_o)$. Crystal data for **1c**·HN_3: $C_{13}H_{28}N_8O_2$, M = 328.4, monoclinic, space group $P2_1/c$, a = 8.710 (1) Å, b = 9.819 (1) Å, c = 21.635 (3) Å, β = 101.97 (1)°, V = 1809.9 (4) Å^3, Z = 4, D_c = 1.205 g cm^{-3}.

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Supplementary Material Available: Atomic coordinates, temperature factors, bond lengths, and bond angles for **1c**·HN_3 (4 pages); structure factors for dimethyldioxo[16]aneN_5·HN_3 (20 pages). Ordering information is given on any current masthead page.

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Studies on the Reactivity of Bicyclomycin with Nucleophilic Amino Acid Derivatives†,1

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Bicyclomycin (1) is a clinically useful antibiotic marketed under the trade name Bicozamycin. Its unique structure and broad spectrum of antimicrobial activity have contributed to the increasing interest in this drug.[2-15] Biochemical studies indicate that 1 binds with select bacterial inner-membrane proteins (i.e., sulfhydryl-containing residues) leading to the disruption of cell wall growth.[5] Controversy exists as to whether drug activation occurs by an initial chemical[6-11] or enzyme-mediated[7,12] process prior to binding with the biological substrate. Recent studies[9-11] in our laboratory on the chemical reactivity of bicyclomycin with simple thiols and amines have provided support for the former scenario. These investigations led to the discovery of an extraordinary transformation in which drug modification proceeded with the generation of piperidinetrione **5** and loss of ammonia (Scheme I).[10,11] The facility of this reaction and the unusual structural properties of **5** led us to speculate that this process may be necessary for complete drug function and that **5** may serve as an efficient trap for additional nucleophiles present at the receptor site.[10,11] In this study, the chemical reactivity of bicyclomycin with nucleophilic amino acid derivatives is examined. We report the *first* examples of the reaction of 1 with cysteine derivatives. Drug modification proceeded rapidly at intermediate pH values to generate sulfide **5**. Special attention is also drawn to the susceptibility of piperidinetrione **5** toward nucleophilic attack by select amino acid derivatives.

Results and Discussion

Treatment of 1 with ethyl mercaptan (**3a**), *N*-acetyl-L-cysteine methyl ester (**3b**), and *N*-acetyl-L-cysteine *N'*-methylamide[16] (**3c**) in 3:1 tetrahydrofuran–water mixtures ("pH" 7.7–8.7) led to the formation of the C(5a)-sulfide adducts **6a–c**, respectively, along with unreacted bicyclomycin.[17,19] The identity of the two cysteine adducts **6b** and **6c** were established by comparison of the ^{1}H and ^{13}C NMR spectral data with that of **6a** (Table I). An X-ray crystallographic structure of **6a** has been previously described.[10] Sulfides **6a–c** reacted with N_α-acetyl-L-lysine *N'*-methylamide (**7**) (methanol, 45 °C, 16–24 h) to yield **9a–c**.[20] The ^{13}C NMR spectra of **9b** and **9c** indicated that the lysine-mediated process proceeded to give essentially a single compound, while a second minor product was detected in the reaction of **6a** with **7**. FAB mass spectra for **9a–c** exhibited a molecular ion peak corresponding to a 1:1 adduct. Important structural information concerning the site of lysine substitution in **9** was derived from careful inspection of the ^{1}H, COSY, and ^{13}C NMR spectra of each product (Table I). Significantly, a resonance is observed in the ^{1}H and COSY spectra for a C(5) methine proton suggesting that the amine-mediated reaction had proceeded at C(6) in **5** (**6**) with cleavage of the C(6)–C(5) bond. In agreement with this contention, the C(6) resonance in **9** appears significantly upfield (Δ ppm ~33) from the corresponding signal in **6**. Comparison of the ^{1}H and ^{13}C NMR spectral data for **6** and **9** indicated that the site of attachment of the triose group to the remaining molecule differs in these two adducts. In the ^{1}H NMR of **9** a downfield shift (Δ ppm ~0.5) of the C(1') methine proton

† Dedicated to the memory of Professor E. T. Kaiser.

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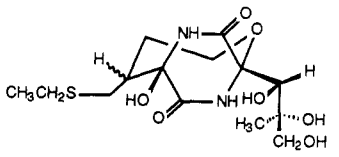
(17) A small amount (0.8 mg, 3%) of the direct C(5a)-substituted ethyl mercaptan adduct i[18] was isolated from reaction of 1 with **3a**.



i

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(19) The amount of bicyclomycin recovered in these reactions ranged from 8 to 27%. A comparable result was observed upon treatment of 1 with **3c** in 9:1 methanol–water mixtures. TLC analysis of the reaction prior to workup indicated the presence of **6c** along with a small amount of 1.
(20) TLC analysis prior to workup indicated that the reaction was complete.

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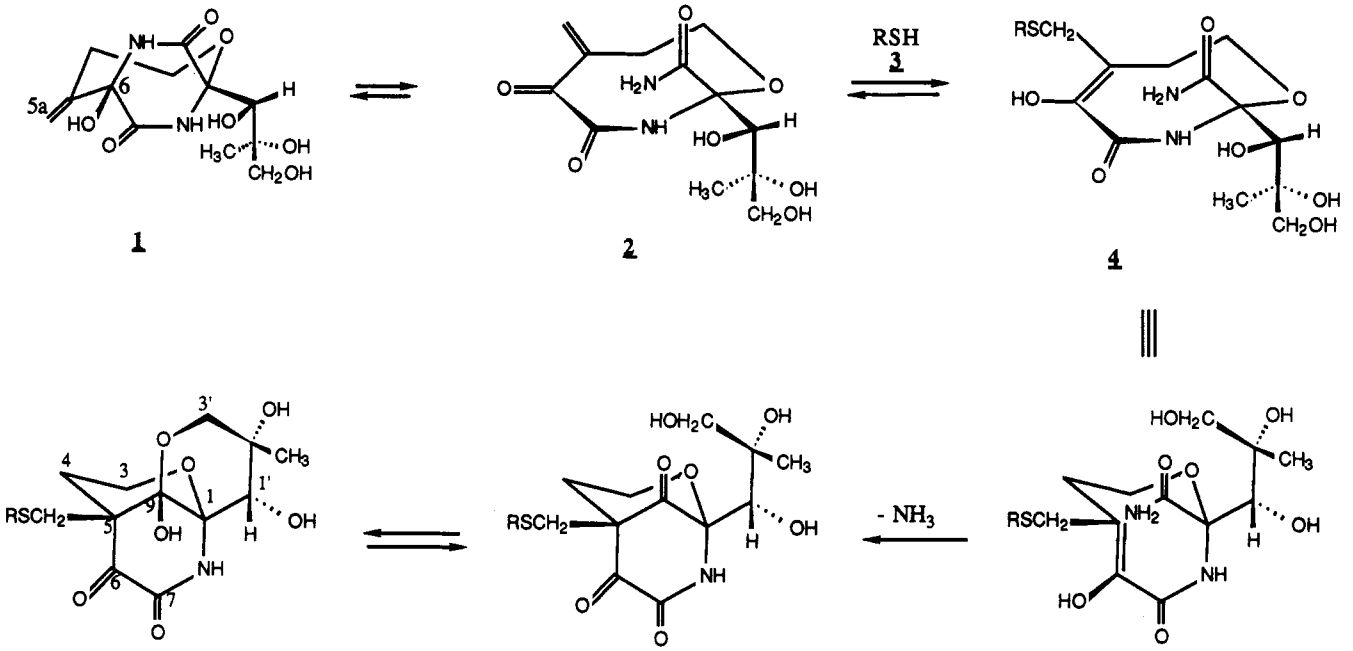
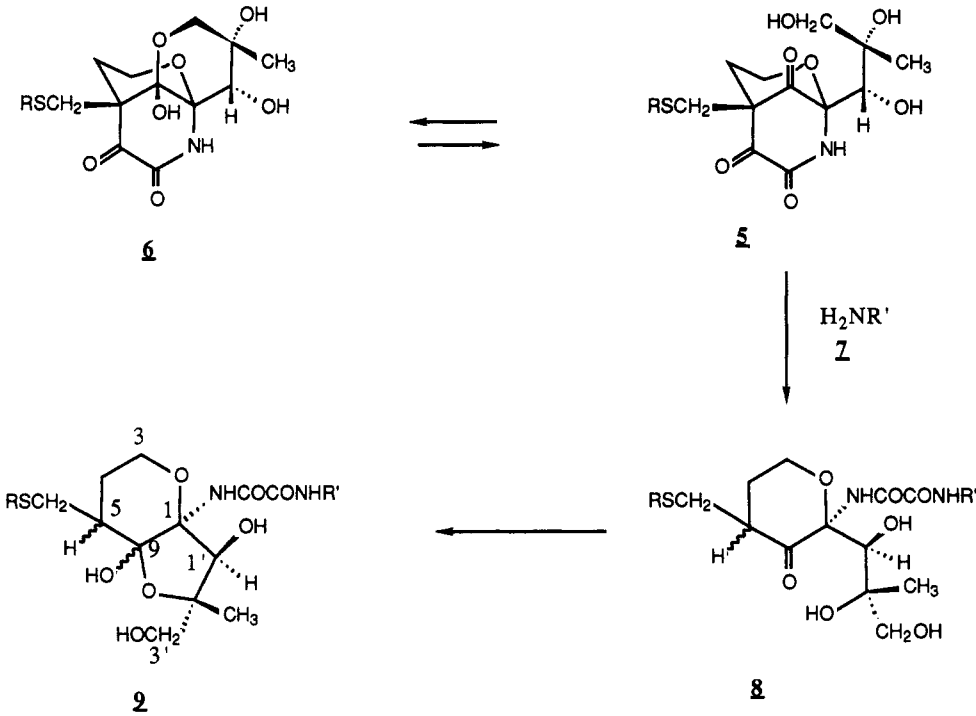
Scheme I. Proposed Mechanism for the Formation of Compounds 6a–c



6 a, R = CH_2CH_3
b, R = $CH_2CH(CO_2CH_3)(NHCOCH_3)$
c, R = $CH_2CH(CONHCH_3)(NHCOCH_3)$

Scheme II. Proposed Mechanism for the Formation of Compounds 9a–c



a, R = CH_2CH_3, R' = $(CH_2)_4CH(CONHCH_3)(NHCOCH_3)$
b, R = $CH_2CH(CO_2CH_3)(NHCOCH_3)$, R' = $(CH_2)_4CH(CONHCH_3)(NHCOCH_3)$
c, R = $CH_2CH(CONHCH_3)(NHCOCH_3)$, R' = $(CH_2)_4CH(CONHCH_3)(NHCOCH_3)$

and an upfield shift (Δ ppm ~0.4) of the C(3′) methylene hydrogens was noted versus **6**. Correspondingly, in the ^{13}C NMR spectrum of **9** the C(2′) resonance occurs downfield (Δ ppm ~14) from **6**, while an upfield shift (Δ ppm ~4) of the C(3′) signal versus **6** is observed. These findings in conjunction with the mass and infrared spectral data are consistent with the formation in these reactions of the C(6)-substituted lysine tetrahydropyranyl adducts **9**.[21]

Table I. Select Physical and Spectral Properties of Functionalized Bicyclomycin Adducts

			^{1}H NMR[c]				^{13}C NMR[d]				
compd no.	yield,[a] %	mp,[b] °C	C(5)H	C(1′)H	C(3′)*H*H′	C(3′)H*H*′	C(1)	C(6)	C(9)	C(2′)	C(3′)
1		188–191		3.89[e] (s)	3.31 (d, 10.9)	3.44 (d, 10.9)	87.74[e]	81.48	166.25	77.01	66.62
6a	45	216–218		3.92 (s)	3.63 (d, 12.3)	4.04 (d, 12.3)	85.33	195.17	96.16	71.95[f]	72.42[f]
6b	31	148–150		3.91 (s)	3.64 (d, 12.2)	4.01 (d, 12.2)	85.49	195.27	95.79	71.93[f]	72.44[f]
6c	23	160		3.92 (s)	3.62 (d, 12.1)	4.02 (d, 12.1)	85.45	195.24	95.88	71.95[f]	72.41[f]
9a	51	125–127	1.35–2.33 (m)	4.45 (s)	3.40–3.52 (m)	3.40–3.52 (m)	91.08	161.96[f]	99.59	85.55	68.11
9b	39	124–125	1.29–2.15 (m)	4.44 (s)	3.40–3.50 (m)	3.40–3.50 (m)	91.12	162.01[f]	99.54	85.63	68.05
9c	39	140–142	1.28–2.15[g] (m)	4.42 (s)	3.40–3.50 (m)	3.40–3.50 (m)	91.10[h]	162.03	99.60	85.66	68.26

[a] Purified yields from immediate precursor. [b] Melting (decomposition) points are uncorrected. [c] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si, followed by the multiplicity of the signal and in select cases the coupling constant(s) in hertz. All spectra were recorded at 300 MHz, and the solvent used was CD_3OD. The ^{1}H NMR assignments were verified from the corresponding COSY spectrum. [d] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si. All spectra were obtained at 75.5 MHz. The solvent used was CD_3OD. [e] Reference 14. [f] This peak may be interchanged with a nearby signal (±1.5 ppm). [g] The ^{1}H NMR assignments were supported by the corresponding relayed COSY and ROESY (200 ms) spectra. [h] The ^{13}C NMR assignments were supported by both the one-bond and the long-range proton detected proton–carbon correlation experiments.

One conceivable pathway for the formation of the bis-substituted bicyclomycin adducts **9** is depicted in Scheme II. Lysine substitution is envisioned to proceed at C(6) in **5** to generate the piperidinetrione ring-cleaved product **8**. Hemiketal bond formation at C(9) in the final step yields the observed product **9**.

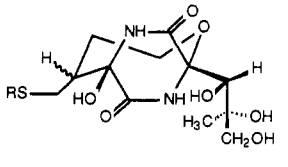
Preliminary information concerning the susceptibility of the C(6)-carbonyl group toward nucleophilic attack was derived from the reaction of sulfide **6b** with **7**. Under the employed conditions, no reaction was observed at the cysteine methyl ester, indicating that the pyruvamide carbonyl system was more prone to attack than the carbomethoxy carbonyl group. The specificity as well as the efficiency of the multiple bicyclomycin binding process was also gauged by treatment of **1** with **3c** (1.3 equiv) and **7** (1.5 equiv) in a 9:1 methanol–water mixture (45 °C, 20 h, final "pH" 9.2). Both **6c** and **9** were observed.[22] These results demonstrated that reaction of bicyclomycin with lysine **7** did not take place until cysteine-mediated activation of the drug to piperidinetrione **5c** had occurred.

Conclusions

The successful reaction of bicyclomycin with both cysteine and lysine residues provides new information concerning the chemical reactivity of the antibiotic with nucleophilic amino acids. The C(5)-cysteine-substituted adducts **6b** and **6c** represent the first reported examples of the interaction of bicyclomycin with sulfhydryl-containing amino acids. This result is important in light of the earlier projections[5] concerning the site of drug binding on the inner-membrane bacterial protein(s). The relative ease in which these sulfide adducts undergo further modification with lysine derivatives provides support for the notion that disruption of bacterial cell wall growth may proceed by a multiple binding process. The generality, mechanism, and implications of these chemical transformations in relation to the biological process are currently being pursued.

(21) The spectral data did not permit the stereochemical assignment of C(5) and C(9) in **9**.

(22) A small amount of the direct C(5a)-substituted cysteine adduct ii was isolated in this transformation. Compound ii does not react with **7** under the employed conditions.[18]



ii, R = $CH_2CH(CONHCH_3)(NHCOCH_3)$

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were taken at 300 and 75 MHz, respectively. The relayed COSY, ROESY (200 ms),[23] one-bond,[24] and long-range[25] proton detected proton–carbon correlation NMR experiments were performed by Dr. Gary E. Martin on a Varian VXR-500 NMR instrument at the Burroughs Wellcome Company. The low and high resolution FAB spectral studies were conducted at the Baylor College of Medicine on a VG ZAB-SEQ instrument by Dr. Simon Gaskell and Mr. Ralph Orkiszewski. pH measurements were determined on a Radiometer pHM26 meter equipped with a Radiometer G202 glass electrode.

***N*-Acetyl-L-cysteine Methyl Ester (3b).**[26] A solution containing *N,S*-diacetyl-L-cysteine methyl ester[27] (100 mg, 0.45 mmol) in 5% aqueous NaOH (1.2 mL, 1.5 mmol) was stirred at room temperature for 45 min. The reaction mixture was acidified with a 0.1 M H_2SO_4 solution and extracted with ether (3 × 10 mL), dried (Na_2SO_4), and concentrated. The crude material was purified by PTLC (silica gel) using 10% methanol–chloroform to yield **3b** (13 mg, 16%) as a semisolid: R_f 0.65 (10% methanol–chloroform); $[\alpha]^{25}_D = -24°$ (c = 1, CH_3OH); IR ($CHCl_3$) 3300, 1750, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 8.90 Hz, 1 H, HS), 2.08 (s, 3 H, $NHCOCH_3$), 3.01 (dd, J = 4.09, 8.90 Hz, 2 H, $HSCH_2$), 3.80 (s, 3 H, $COOCH_3$), 4.87–4.93 (m, 1 H, CH_2CH), 6.62 (d, J = 3.23 Hz, 1 H, NH); ^{13}C NMR ($CDCl_3$) 22.98 ($NHCOCH_3$), 26.73 ($HSCH_2$), 52.67, 53.50 ($CH_2CHCOOCH_3$), 169.87, 170.56 ($NHCOCH_3$, $COOCH_3$) ppm; M_r 177.04626 (calcd for $C_6H_{11}NO_3S$, 177.04597).

***N*-Acetyl-L-cysteine *N*′-Methylamide (3c).** A solution containing *N,S*-diacetyl-L-cysteine methyl ester[27] (400 mg, 1.8 mmol) in 10% aqueous methylamine (4 mL, 13 mmol) was stirred at room temperature for 50 min. The solvent was removed in vacuo and the residue was recrystallized from ethanol to yield **3c** (120 mg, 30%): R_f 0.50 (10% methanol–chloroform); mp 196–197 °C (lit.[16] mp 196–199 °C); $[\alpha]^{25}_D = -31.6°$ (c = 1, CH_3OH), $[\alpha]^{25}_D = -21.7°$ (c = 1, H_2O) (lit.[16] $[\alpha]^{25}_D = -27.9°$ (c = 1, H_2O)); IR (KBr) 3270, 1630 cm^{-1}; ^{1}H NMR (CD_3OD) δ 2.01 (s, 3 H, $NHCOCH_3$), 2.73 (s, 3 H, NCH_3), 2.73–2.88 (m, 2 H, $HSCH_2$), 4.42 (t, J = 6.29 Hz, 1 H, CH_2CH); ^{13}C NMR (CD_3OD) 22.52 ($NHCOCH_3$), 26.33, 26.80 ($HSCH_2CH$, $NHCH_3$), 57.16 ($HSCH_2CH$), 172.84, 173.40, ($CONHCH_3$, $NHCOCH_3$) ppm; MS (FAB) 177 [M + 1]$^+$.

Reactions of Bicyclomycin (1) with Thiols. General Procedure for the Preparation of Compounds 6a–c. A solution of **1** and **3** (1.1–1.6 equiv) in THF/H_2O (3:1, "pH" 7.7–8.7) was stirred for 24–48 h at room temperature. The solvent was removed in vacuo and the residue was purified by PTLC (silica gel) using 10% methanol–chloroform as the eluent.

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Compound **6a**. Using **1** (25 mg, 0.083 mmol) and **3a** (0.1 mL, 1.35 mmol) in THF/H_2O (3:1, 3 mL, "pH" 7.7–8.6) at room temperature (24 h) gave 13 mg (45%) of **6a**: R_f 0.70 (10% methanol–chloroform); mp 216–218 °C; $[\alpha]^{25}_D$ = +51.7° (c = 1, CH_3OH); IR (KBr) 1730, 1670 cm^{-1}; ^1H NMR (CD_3OD) δ 1.15 (s, 3 H, C(2')CH_3), 1.25 (t, J = 7.27 Hz, 3 H, CH_2CH_3), 1.91 (br d, J = 14.00 Hz, 1 H, C(4)HH'), 2.31 (dt, J = 6.30, 14.00 Hz, 1 H, C(4)HH'), 2.58 (q, J = 7.27 Hz, 2 H, CH_2CH_3), 2.90 ($^1/_2$ AB q, J = 13.93 Hz, 1 H, C(5a)HH'), 3.02 ($^1/_2$ AB q, J = 13.93 Hz, 1 H, C(5a)HH'), 3.63 (d, J = 12.28 Hz, 1 H, C(3')HH'), 3.76 (dt, J = 2.10, 14.00 Hz, 1 H, C(3)HH'), 3.92 (s, 1 H, C(1')H), 4.03 (dd, J = 6.30, 14.00 Hz, 1 H, C(3)HH'), 4.04 (d, J = 12.28 Hz, 1 H, C(3')HH'); ^{13}C NMR (CD_3OD) 15.03 (CH_2CH_3), 21.14 (C(2')CH_3), 29.58 (CH_2CH_3), 32.40 (C(5a)), 33.15 (C(4)), 56.75 (C(5)), 58.68 (C(3)), 70.88, 71.95, 72.42 (C(1'), C(2'), C(3')), 85.33 (C(1)), 96.16 (C(9)), 159.99 (C(7)), 195.17 (C(6)) ppm; M_r 347.1053 (calcd for $C_{14}H_{21}NO_7S$, 347.1039).

Compound **6b**. Using **1** (60 mg, 0.198 mmol) and **3b** (40 mg, 0.226 mmol) in THF/H_2O (3:1, 9 mL, "pH" 8.2–8.4) at room temperature (48 h) under argon gave 28 mg (31%) of **6b**: R_f 0.60 (10% methanol–chloroform); mp 148–150 °C; IR (KBr) 3330, 1735, 1690, 1660 cm^{-1}; ^1H NMR (CD_3OD) δ 1.15 (s, 3 H, C(2')CH_3), 1.94 (dd, J = 2.19, 13.79 Hz, 1 H, C(4)HH'), 2.00 (s, 3 H, NHCOCH_3), 2.35 (dt, J = 6.49, 13.79 Hz, 1 H, C(4)HH'), 2.87 (d, J = 13.90 Hz, 1 H, C(5a)HH'), 2.87 (dd, J = 8.25, 13.85 Hz, 1 H, SCHH'CH), 3.02 (dd, J = 5.10, 13.85 Hz, 1 H, SCHH'CH), 3.07 (d, J = 13.90 Hz, 1 H, C(5a)HH'), 3.64 (d, J = 12.20 Hz, 1 H, C(3')HH'), 3.68–3.79 (m, 1 H, C(3)HH'), 3.74 (s, 3 H, $COOCH_3$), 3.91 (s, 1 H, C(1')H), 4.01 (d, J = 12.20 Hz, 1 H, C(3')HH'), 3.99–4.05 (m, 1 H, C(3)HH'), 4.62 (dd, J = 5.10, 8.25 Hz, 1 H, SCH_2CH); ^{13}C NMR (CD_3OD) 21.17 (C(2')CH_3), 22.35 (NHCOCH_3), 32.42, 33.16 (C(4), C(5a)), 37.27 (SCH_2CH), 52.97, 53.72 (SCH_2CH, $COOCH_3$), 57.10 (C(5)), 58.68 (C(3)), 70.92, 71.93, 72.44 (C(1'), C(2'), C(3')), 85.49 (C(1)), 95.79 (C(9)), 159.98 (C(7)), 172.63, 173.38 (NHCOCH$_3$, $COOCH_3$), 195.27 (C(6)) ppm; M_r (FAB) 463.13897 [M + 1]$^+$ (calcd for $C_{18}H_{27}N_2O_{10}S$, 463.13864).

Compound **6c**. Using **1** (22 mg, 0.073 mmol) and **3c** (15 mg, 0.085 mmol) in THF/H_2O (3:1, 3 mL, "pH" 8.4–8.7) at room temperature (48 h) under argon gave 7.5 mg (23%) of **6c**: R_f 0.55 (10% methanol–chloroform); mp 160 °C; IR (KBr) 3280, 1690, 1640 cm^{-1}; ^1H NMR (CD_3OD) δ 1.15 (s, 3 H, C(2')CH_3), 1.95 (dd, J = 2.50, 13.77 Hz, 1 H, C(4)HH'), 2.01 (s, 3 H, NHCOCH_3), 2.36 (dt, J = 6.49, 13.77 Hz, C(4)HH'), 2.74 (s, 3 H, NCH_3), 2.79 (dd, J = 8.50, 13.80 Hz, 1 H, SCHH'CH), 2.89 (d, J = 13.84 Hz, 1 H, C(5a)HH'), 2.97 (dd, J = 5.56, 13.80 Hz, 1 H, SCHH'CH), 3.07 (d, J = 13.84 Hz, 1 H, C(5a)HH'), 3.62 (d, J = 12.12 Hz, 1 H, C(3')HH'), 3.73 (dt, J = 2.50, 13.55 Hz, 1 H, C(3)HH'), 3.92 (s, 1 H, C(1')H), 3.98–4.04 (m, 1 H, C(3)HH'), 4.02 (d, J = 12.12 Hz, 1 H, C(3')HH'), 4.46 (dd, J = 5.56, 8.50 Hz, 1 H, SCH_2CH); ^{13}C NMR (CD_3OD) 21.15 (C(2')CH_3), 22.56 (NHCOCH_3), 26.44 ($NHCH_3$), 32.61, 33.25 (C(4), C(5a)), 37.76 (SCH_2CH), 54.52 (SCH_2CH), 57.01 (C(5)), 58.67 (C(3)), 70.94, 71.95, 72.41 (C(1'), C(2'), C(3')), 85.45 (C(1)), 95.88 (C(9)), 159.97 (C(7)), 173.24, 173.42 (NHCOCH$_3$, CONHCH$_3$), 195.24 (C(6)) ppm; M_r (FAB) 462.15391 [M + 1]$^+$ (calcd for $C_{18}H_{28}N_3O_9S$, 462.15463).

Reactions of 6 with N_α-Acetyl-L-lysine N'-Methylamide (7). General Procedure for the Preparation of Compounds 9a–c. A solution of **6a–c** and **7** (1.4–2.1 equiv) in methanol ("pH" 9.3–9.5) was stirred at 45 °C for 16–24 h. The solvent was removed in vacuo and the residue was purified by PTLC (silica gel) using methanol–chloroform as the eluent.

Compound **9a**. Use of **6a** (40 mg, 0.115 mmol) and **7** (49 mg, 0.244 mmol) in methanol (5 mL, "pH" 9.3) at 45 °C (16 h) gave 32 mg (51%) of **9a**: R_f 0.40 (10% methanol–chloroform); mp 125–127 °C; IR (KBr) 3320, 2920, 1650 cm^{-1}; ^1H NMR (CD_3OD) δ 1.20–1.28 (m, 6 H, SCH_2CH_3, C(2')CH_3), 1.35–2.33 (m, 9 H, $HNCH_2(CH_2)_3$, C(4)HH', C(5)H), 1.99 (s, 3 H, NHCOCH_3), 2.47–2.67 (m, 3 H, SCH_2CH_3, C(5a)HH'), 2.72 (s, 3 H, NCH_3), 2.98–3.17 (m, 1 H, C(5a)HH'), 3.20–3.35 (m, $HNCH_2(CH_2)_3$, CD_3OD), 3.40–3.52 (m, 2 H, C(3')HH'), 3.57–3.94 (m, 2 H, C(3)HH'), 4.24 (dd, J = 5.32, 8.62 Hz, 1 H, $HN(CH_2)_4CH$), 4.45 (s, 1 H, C(1')H); ^{13}C NMR (CD_3OD) 15.17 (CH_2CH_3), 18.76 (C(2')CH_3), 22.52 ($NHCOCH_3$), 24.13 ($HNCH_2CH_2CH_2CH_2CH$), 25.07 (C(4)), 26.33, 26.78 (NCH_3, CH_2CH_3), 29.67, 29.86 ($HNCH_2CH_2CH_2CH_2CH$, C(5a)), 32.57 ($HNCH_2CH_2CH_2CH_2CH$), 40.47, 40.91 ($HNCH_2(CH_2)_3CH$, C(5)), 54.73 ($HN(CH_2)_4CH$), 58.07 (C(3)), 68.11 (C(3')), 78.60 (C(1')), 85.55 (C(2')), 91.08 (C(1)), 99.59 (C(9)), 161.18, 161.96 (COCO), 173.37, 174.93 (NHCOCH$_3$, CONHCH$_3$) ppm; MS (+FAB) 549 [M + 1]$^+$.

Compound **9b**. Use of **6b** (28 mg, 0.060 mmol) and **7** (24 mg, 0.120 mmol) in methanol (5 mL, "pH" 9.3) at 45 °C (24 h) gave 15.5 mg (39 %) of **9b**: R_f 0.45 (15% methanol–chloroform); mp 124–125 °C; IR (KBr) 3240, 2940, 1740, 1660 cm^{-1}; ^1H NMR (CD_3OD) δ 1.24 (s, 3 H, C(2')CH_3), 1.29–2.15 (m, 9 H, $HNCH_2(CH_2)_3CH$, C(4)HH', C(5)H), 1.99, 2.00 (2 s, 6 H, 2 × NHCOCH_3), 2.66–3.06 (m, 4 H, C(5a)HH', SCH_2CH), 2.72 (s, 3 H, NCH_3), 3.24–3.34 (m, $HNCH_2(CH_2)_3CH$, CD_3OD), 3.40–3.50 (m, 2 H, C(3')HH'), 3.56–4.00 (m, 2 H, C(3)HH'), 3.74 (s, 3 H, $COOCH_3$), 4.24 (dd, J = 5.37, 8.38 Hz, $HN(CH_2)_4CH$), 4.44 (s, 1 H, C(1')H), 4.64–4.67 (m, 1 H SCH_2CH); ^{13}C NMR (CD_3OD) 18.73 (C(2')CH_3), 22.38, 22.52 (2 × NHCOCH_3), 24.15 ($HNCH_2CH_2CH_2CH_2CH$), 25.03 (C(4)), 26.32 (NCH_3), 29.66, 30.85 ($HNCH_2CH_2CH_2CH_2CH$, C(5a)), 32.59 ($HNCH_2CH_2CH_2CH_2CH$), 34.45 (SCH_2CH), 40.49, 40.97 ($HNCH_2(CH_2)_3CH$, C(5)), 52.97 ($COOCH_3$), 53.65, 54.74 ($HN(CH_2)_4CH$, SCH_2CH), 58.18 (C(3)), 68.05 (C(3')), 78.59 (C(1')), 85.63 (C(2')), 91.12 (C(1)), 99.54 (C(9)), 161.23, 162.01 (COCO), 172.60, 173.30, 173.39, 174.94 ($COOCH_3$, 2 × NHCOCH$_3$, CONHCH$_3$) ppm; MS (+FAB) 664 [M + 1]$^+$.

Compound **9c**. Use of **6c** (50 mg, 0.108 mmol) and **7** (30 mg, 0.149 mmol) in methanol (7 mL, "pH" 9.5) at 45 °C (24 h) gave 28 mg (39%) of **9c**: R_f 0.40 (15% methanol–chloroform); mp 140–142 °C; IR (KBr) 3360, 2900, 1650 cm^{-1}; ^1H NMR (CD_3OD) δ 1.24 (s, 3 H, C(2')CH_3), 1.28–2.15 (m, 9 H, $HNCH_2(CH_2)_3CH$, C(4)HH', C(5)H), 1.98, 1.99 (2 s, 6 H, 2 × NHCOCH_3), 2.63–3.06 (m, 4 H, C(5a)HH', SCH_2CH), 2.71, 2.74 (2 s, 6 H, 2 × NCH_3), 3.22–3.37 (m, $HNCH_2(CH_2)_3CH$, CD_3OD), 3.40–3.50 (m, 2 H, C(3')HH'), 3.65–3.93 (m, 2 H, C(3)HH'), 4.23 (dd, J = 5.46, 8.62 Hz, 1 H, $HN(CH_2)_4CH$), 4.42 (s, 1 H, C(1')H), 4.48 (dd, J = 4.92, 8.76 Hz, 1 H, SCH_2CH); ^{13}C NMR (CD_3OD) 18.78 (C(2')CH_3), 22.62 (2 × NHCOCH_3), 24.18 ($HNCH_2CH_2CH_2CH_2CH$), 25.21 (C(4)), 26.34, 26.51 (2 × NCH_3), 29.68 ($HNCH_2CH_2CH_2CH_2CH$), 30.84 (C(5a)), 32.65 ($HNCH_2CH_2CH_2CH_2CH$), 35.08 (SCH_2CH), 40.54 ($HNCH_2CH_2CH_2CH_2CH$), 40.94 (C(5)), 54.30 (SCH_2CH), 54.78 ($HN(CH_2)_4CH$), 58.20 (C(3)), 68.26 (C(3')), 78.74 (C(1')), 85.66 (C(2')), 91.10 (C(1)), 99.60 (C(9)), 161.29 (HNCOCONH-$(CH_2)_4$), 162.03 (HNCOCONH$(CH_2)_4$), 173.38, 174.94 (2 × CONHCH$_3$, 2 × NHCOCH$_3$) ppm; MS (+FAB) 685 [M + Na]$^+$; M_r 685.2885 (calcd for $C_{27}H_{46}N_6O_{11}SNa$, 685.2945).

Reaction of 1 with N-Acetyl-L-cysteine N'-Methylamide (3c) and N_α-Acetyl-L-lysine N'-Methylamide (7). A solution of **1** (20 mg, 0.066 mmol), N-acetyl-L-cysteine N'-methylamide (**3c**) (15 mg, 0.085 mmol), and N_α-acetyl-L-lysine N'-methylamide (**7**) (20 mg, 0.099 mmol) in methanol–water (9:1, 6 mL, "pH" 9.2) was stirred at 45 °C under argon (40 h). The solvent was removed in vacuo and the residue was purified by PTLC (silica gel) using 12% methanol–chloroform (three developments) as the eluent to yield **6c** (1 mg) and **9c** (3 mg). Compound **9c** was further purified by PTLC (silica gel) using 10% methanol–chloroform (three developments) as the eluent to give 2 mg of pure material: R_f 0.40 (15% methanol–chloroform); mp 140 °C; ^1H NMR (CD_3OD) δ 1.24 (s, 3 H, C(2')CH_3), 1.28–2.15 (m, 9 H, $HNCH_2(CH_2)_3CH$, C(4)HH', C(5)H), 1.98, 1.99 (2 s, 6 H, 2 × NHCOCH_3), 2.63–3.06 (m, 4 H, C(5a)HH', SCH_2CH), 2.71, 2.74 (2 s, 6 H, 2 × NCH_3), 3.22–3.37 (m, $HNCH_2(CH_2)_3CH$, CD_3OD), 3.40–3.50 (m, 2 H, C(3')HH'), 3.65–3.93 (m, 2 H, C(3)HH'), 4.23 (dd, J = 5.46, 8.62 Hz, 1 H, $HN(CH_2)_4CH$), 4.42 (s, 1 H, C(1')H), 4.48 (dd, J = 4.92, 8.76 Hz, 1 H, SCH_2CH).

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